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1 Ectomycorrhizal fungal communities in urban parks are similar to those in natural forests but
2 shaped by vegetation and park age

3

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9 Running Head: Ectomycorrhizal fungal communities in urban parks

10

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12

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14 **Abstract**

15 Ectomycorrhizal (ECM) fungi are important mutualists for growth and health of most boreal
16 trees. Forest age and its host species composition can impact the composition of ECM fungal
17 communities. Although plentiful empirical data exist for forested environments, the effects of
18 established vegetation and its successional trajectories on ECM fungi in urban greenspaces
19 remain poorly understood. We analyzed ECM fungi in 5 control forests and 41 urban parks of
20 two plant functional groups (conifer and broadleaf trees) and in three age categories (10, ~50
21 and >100 years old) in southern Finland. Our results show that although ECM fungal richness
22 was marginally greater in forests than in urban parks, urban parks still hosted rich and diverse
23 ECM communities. ECM community composition differed between the two habitats, but was
24 driven by taxon rank order reordering, as key ECM taxa remained largely the same. In parks,
25 the ECM communities differed between conifer and broadleaf trees. The successional
26 trajectories of ECM fungi – as inferred in relation to the time since park construction –
27 differed among the conifers and broadleaf trees: the ECM fungal communities changed over
28 time under the conifers, whereas communities under broadleaf trees provided no evidence for
29 such age related effects. Our data show that plant-ECM interactions in urban parks, in spite of
30 being constructed environments, are surprisingly similar in richness to those in natural forests.
31 This suggests that the presence of host trees, rather than soil characteristics or even
32 disturbance regime of the system, determine ECM fungal community structure and diversity.

33 **Importance**

34 In urban environments soil and trees improve environmental quality and provide essential
35 ecosystem services. Ectomycorrhizal (ECM) fungi enhance plant growth and performance,
36 increasing plant nutrient acquisition and protecting plants against toxic compounds. Recent
37 evidence indicates that soil-inhabiting fungal communities - including ECM and saprotrophic
38 fungi - in urban parks are affected by plant functional type and park age. However, ECM
39 fungal diversity and its responses to urban stress, plant functional type or park age remain

40 unknown. The significance of our study is in identifying - in greater detail – the responses of
41 ECM fungi in the rhizospheres of conifer and broadleaf trees in urban parks. This will greatly
42 enhance our knowledge of ECM fungal communities under urban stresses, and can be utilized
43 by urban planners to improve urban ecosystem services.

44 **Introduction**

45

46 Soils and trees in urban parks improve environmental quality and provide essential ecosystem
47 services (1). Healthy urban trees facilitate rainwater storage, mitigate urban heat island
48 effects, support biodiversity, and provide an aesthetically appealing environment for urban
49 residents (2). Ectomycorrhizal (ECM) fungi, necessary mutualists of most boreal trees, are
50 important for host performance as well as for nutrient cycling at an ecosystem level (3). ECM
51 fungi enhance plant growth and performance (4), increase plant nutrient acquisition (5) and
52 protect plants against toxic compounds (6).

53

54 In urban parks, soil organisms are subject to anthropogenic disturbances, such as pollution,
55 fertilization, trampling and the removal of plant litter. ECM are sensitive to urban disturbance
56 (7) and their richness and abundance lower in urban ecosystems compared to rural areas (8,
57 9). Therefore, ECM fungi may be useful indicators reflecting the disturbance status of the
58 below- and aboveground communities in urban areas, particularly where disturbances
59 influence both soil properties and plant health. Although many factors influence ECM fungal
60 community composition in boreal forest ecosystems such as edaphic factors, host plant
61 species composition and stand age (10-12), factors that impact urban ECM fungi and their
62 succession remain unclear (9).

63

64 Most ECM species typically have broad plant host ranges (13) and different hosts associate
65 with divergent ECM fungal communities (14). ECM fungal communities can differ among
66 urban and non-urban trees (9, 15), even among conspecific hosts (16). Recent evidence
67 indicates that soil-inhabiting fungal communities - including ECM and saprotrophic fungi - in
68 urban parks are affected by plant functional type and park age (17). However, ECM fungal
69 diversity and its responses to urban stress, plant functional type or park age remain unknown.

70 In the current study, we extracted ECM fungal sequence data from a broader dataset analyzed
71 in Hui et al. (17). This allowed for exclusive analyses ECM fungal responses in the
72 rhizospheres of conifer and broadleaf trees in 41 parks in the cities of Lahti and Helsinki,
73 Finland. The selected sites represent different park ages (*i.e.*, time since park construction)
74 and thus provide a means to dissect how ECM fungal communities are modified over time in
75 an urban environment. To compare these park communities to those in a more natural and less
76 disturbed environment, we included 5 minimally disturbed rural forests dominated by *Picea*
77 *abies* and *Tilia cordata* as non-urban controls.

78
79 Here, we focus on 1) the response of ECM fungal communities to plant functional type and
80 park age in urban park soils; and 2) potential differences in ECM fungal communities
81 between non-urban control forests and disturbed urban parks (land-use type). Further, we
82 investigated 3) which ECM fungal genera are particularly responsive to land-use, plant
83 functional type, and park age. We hypothesized that: i) ECM fungal communities under
84 conifer and broadleaf trees in urban parks differ from those in control forests. This is because
85 urban soils often have high pH, high concentrations of organic and inorganic pollutants (18)
86 and their microbial communities may be affected by urban management (9). We also
87 predicted that in the forest soil, ECM fungi are more diverse than in urban park soils. This is
88 because of the positive relationship between canopy tree diversity and ECM diversity (9, 11).
89 ii) ECM fungal community structure in urban parks depend on plant functional type. This is
90 because plant functional types differ fundamentally in terms of effects on soil properties (18),
91 allocation of recent photosynthate (19), litter and root exudates (14), upon which ECM fungi
92 depend. iii) ECM fungal communities respond to park age. This is due to the different
93 abilities of early- and late-stage fungi to form symbioses with host roots (20).

94 95 **Results**

96
97 Comparisons of ECM fungal communities between urban parks and control forests
98
99 The control forests and old parks in Lahti, representing roughly similarly aged trees, differed
100 in ECM fungal diversity. Diversity was generally greater in control forests than in the old
101 parks (Fig. 1, Table S1). OTU richness and diversity were lower under conifer trees than
102 broadleaf trees both in old parks and control forests (Fig. 1a, b), whereas evenness showed an
103 opposite trend (Fig. 1c). OTU richness, diversity and evenness correlated positively with soil
104 OM. Soil pH correlated with ECM fungal community richness positively and with evenness
105 negatively (Table S1).
106
107 ECM fungal community composition differed between (i) the old parks and control forests (r^2
108 = 0.425, $p < 0.001$) and (ii) the two tree functional types ($r^2 = 0.587$, $p < 0.001$; Fig. 2a). ECM
109 OTUs were classified into 51 genera throughout the dataset. *Inocybe* was the most dominant
110 genus (13.8% of the ECM fungal sequences, 49 OTUs), followed by *Cenococcum* (11.7%, 21
111 OTUs) and *Wilcoxina* (10.1%, 4 OTUs). To explore the ECM fungal community distinctions
112 between control forests and old parks in Lahti, we conducted GLMM analyses on the ten
113 most abundant genera. Five genera (*Amphinema*, *Piloderma*, *Russula*, *Tomentella* and
114 *Tylospora*) were more abundant in control forests than in old parks (Fig. 3, Table S1), while
115 none of the most abundant genera occurred more frequently in the parks. *Cenococcum* and
116 *Cortinarius* were constantly more abundant under broadleaf trees than conifer, whereas
117 *Wilcoxina* showed an opposite trend. *Russula* and *Tylospora* showed significant plant
118 functional type x land-use type (control forest vs. old Lahti parks) interactions. In addition to
119 these analyses, we also included a set of environmental variables in the GLMM analyses: four
120 ECM genera were correlated with soil N (one positively and three negatively), three with soil
121 C (one positively and two negatively), three with soil OM (all positively), four with

percentage sand (two positively and two negatively) and three with soil pH (two positively and one negatively) (Table S1).

Effects of plant functional group and park age on ECM fungi in urban parks

In parks, ECM OTU richness and evenness had significant plant functional group x park age interactions (Table S2). In young parks, both ECM fungal richness and diversity were indistinguishable between the two plant functional groups. However, ca. 50 years after park establishment, soils under broadleaf trees tended to host more diverse ECM fungal communities than under conifer trees (Fig. 1 a, b). Diversity and evenness of the ECM fungal communities in parks were negatively correlated with soil pH. In our case, all soils were acidic with maximum pH ~ 6.9 (18). As a result, the diversity and evenness declined as pH approached neutral. All diversity indices correlated negatively with soil C (Table S2).

ECM fungal community composition differed clearly between conifer and broadleaf trees in old parks in Lahti. As a result, we conducted analyses separately for the two tree functional groups in parks: ECM fungal communities under broadleaf trees responded to park age ($r^2 = 0.110$, $p = 0.029$, Fig. 2b), but this was not the case under conifer trees ($r^2 = 0.041$, $p = 0.697$).

To study the effects of plant functional type and park age on common ECM fungi in parks, we analyzed the ten most abundant ECM fungal genera using GLMM. The abundances of *Inocybe*, *Wilcoxina* and *Cenococcum* responded similarly and the differences among the two plant functional types became more pronounced in intermediate and old parks than in young parks (Fig. 4). In intermediate and old parks, *Wilcoxina* was more abundant under conifer trees than under broadleaf trees, whereas *Cenococcum* and *Tuber* showed the opposite trend. *Tuber* was also highly abundant under conifer trees in young parks but not under broadleaf

148 trees. *Hebeloma* and *Tomentella* abundances differed across park age, with a higher count in
149 young parks than in intermediate and old parks. *Laccaria* and *Cortinarius* were consistently
150 more abundant under broadleaf trees than under conifer trees, especially in old parks.
151 *Cenococcum*, *Scleroderma* and *Tomentella* showed plant functional type x park age
152 interactions (Table S2). Our GLMM results showed that six ECM fungal genera were
153 correlated with soil N (all negatively), five with C (four positively and one negatively), three
154 with OM (all negatively), seven with percentage sand (one positively and six negatively), and
155 two with pH (one positively and one negatively) (Table S2).

156

157 Discussion

158

159 Our previous research showed that vegetation and park age drive changes in soil properties in
160 urban parks (18) leading to distinct microbial communities (bacteria and fungi) (17). Here, we
161 focused exclusively on ECM fungi and addressed how they respond to land-use type (forest
162 vs urban park), plant functional type and park age under northern climatic conditions. Since
163 different fungal groups have distinct life history strategies (21), we expected that ECM
164 responses would differ from those of the general soil-inhabiting fungi (17).

165

166 Differences in ECM fungal communities between old parks and forests

167

168 Urbanization likely has negative effects on soil properties (18), microbial communities (22)
169 and soil fauna (23). Our data indicate that ECM OTU richness and diversity were greater in
170 control forests than in urban parks, supporting our first hypothesis and corroborating previous
171 observations. Urban anthropogenic disturbance can reduce ECM diversity and richness (8, 9,
172 24, 25), particularly in boreal regions where these fungi are most diverse. Our data are in
173 contrast to predictions that increasing urbanization and the concomitant loss of natural forests

174 will lead to the dramatic suppression of ECM fungi in urbanized ecosystems (22). Instead, we
175 conclude that boreal hosts recruit quite diverse ECM fungi in urban greenspaces, suggesting
176 ECM fungal community resistance and resilience to urbanization and co-occurring
177 anthropogenic disturbances.

178

179 Despite the possible resistance and resilience, the ECM fungal communities in urban parks
180 and control forests differed. This result was driven by taxon rank reordering, not taxon
181 replacement. Urban disturbances (litter removal and raking, trampling, and mowing) are
182 absent in forests, which likely result in alterations between the relative proportions of ECM
183 taxa between urban park and forest communities (9). Furthermore, unlike natural boreal
184 forests typified by podzol soils with organic matter layer developed on top of the soil, urban
185 parks - even the oldest ones in our study - lacked such a clear pedogenesis. The direct and
186 indirect effects of pedogenesis on soil physical-chemical parameters are factors that likely
187 affect ECM fungal communities between the two land-use types. However, despite the
188 absence of visible pedogenesis in the urban settings, ECM fungal richness and diversity were
189 surprisingly similar between the natural and urban environments.

190

191 At the genus level, the ten most abundant ECM genera were present in both urban parks and
192 forest stands, but the abundance of many of these genera differed between urban parks and
193 forests. These observations are in line with previous reports (9). For example, the abundance
194 of *Russula* and *Tylospora* were low in urban parks compared to control forests. This is in
195 accordance with Hartmann et al. who showed that the abundance of *Russula* is negatively
196 related with soil compaction (26). *Tylospora* occurs in decaying wood (27) which is scarce or
197 absent in urban parks. *Tuber* was more frequent in urban parks than in control forests. This
198 result is in agreement with (28) who predicted that *Tuber* may be “pre-adapted” to
199 environmental conditions associated with human activities. *Tuber* species tend to prefer

200 alkaline soils (28). A potential explanation for the observed greater abundance is that the
201 acidic soil in control forest largely suppresses *Tuber* species, while they may survive in the
202 neutral or weakly acid soils, which indeed typify urban environments (29).

203

204 Generally our results suggest that ECM fungal richness and diversity were comparable in
205 urban parks and control forests, albeit minimally different. ECM fungal communities shared a
206 number of taxa between the land-use types, but were reordered and dominants replaced as
207 indicated by our genus level analyses.

208

209 Effects of plant functional type on ECM fungal communities in parks

210

211 Supporting previous findings (17, 30) and our second hypothesis, the two tree functional
212 groups hosted distinct ECM fungal communities – both in their diversity and composition.
213 Reasons for the observed compositional differences may lie in the plant-ECM fungus
214 interaction. Plant functional types can influence ECM fungal communities in several ways,
215 including effects through host specificity, modulation of edaphic conditions, litter quality and
216 quantity (labile or recalcitrant), and rhizodeposition (root exudates) (14, 31-33). A recent
217 meta-analysis revealed that host family explained 34% of the variation in ECM fungal
218 community composition (34). This may be a result of specific molecular signaling between
219 the host and its fungal symbionts that determines their compatibility (35).

220

221 Our previous studies show that plant functional types modify soils differently and soils under
222 conifer trees have lower pH, but higher %OM, %N and %C than soils under broadleaf trees in
223 urban parks (18). In the current study, we found that six of the 10 most abundant ECM fungal
224 genera in urban parks were negatively correlated with soil N. Nitrogen content is a major
225 factor influencing ECM communities (35). High N supply suppresses biomass of ECM fungi,

226 because when the host tree can easily obtain nutrients, there is no need to form such plant-
227 fungi symbiont (36, 37). However these chemical responses and correlations did not
228 remarkably affect ECM fungal community richness and diversity.

229

230 Differences in the ECM fungal communities between the two tree functional types were
231 largely attributable to shifts in the abundances of some ECM fungal genera. For example,
232 *Cenococcum* was more common with broadleaf trees than conifers. Twieg et al. showed that
233 the mean relative abundance of *Cenococcum* on broadleaf tree roots (paper birch, *Betula*
234 *papyrifera*) was about four times greater than on conifer trees (Douglas-fir) in a mixed
235 temperate forest (12). *Cenococcum* – one of the most common ECM species in boreal forest
236 soil – seems to respond negatively to high nitrogen in the soil (11). *Cenococcum* is common
237 in soils with low nitrogen content, thus, as a result of the N deposition and subsequent higher
238 N availability, the taxon declines (38, 39). Indeed, in our study, *Cenococcum* was negatively
239 correlated with N in park soils that accumulate traffic-derived nitrogen (40). *Wilcoxina* spp.,
240 in turn, are generalists and well adapted to a wide range of plant community types. They are
241 often among the dominant ECM taxa in coniferous forest (41, 42). Similarly, in parks,
242 *Wilcoxina* spp. were clearly associated with conifer trees. These results highlight that, despite
243 the distinct environmental conditions in parks and forests, ECM fungus host preferences
244 operate similarly regardless of land use. This highlights the pivotal role of plant identity in
245 controlling plant-fungus symbiosis.

246

247 The effects of park age on the ECM fungal community

248

249 ECM fungal diversity seemed rather insensitive to park age. This finding supports neither our
250 third hypothesis nor previous results by Twieg et al., who showed that ECM diversity
251 increased as stand age increased in soils under conifer trees in natural forests (12).

252 Presumably ECM diversity would increase as plants grow older, because trees in young parks
253 lack an extensive root system for ECM colonization (43). Further, young soils may lack an
254 extensive ECM propagule bank because landfill top soils are common in park construction
255 and because of insufficient propagule dispersal to recently established habitats (44). The
256 relatively stable ECM diversity that we observed across park ages may result from the
257 minimal competition in the urban environments allowing many ECM fungi to rapidly
258 colonize roots of young trees. It appears that young trees are equally suitable hosts for these
259 ECM fungal spores to colonize their roots. Although parks soil characteristics change by age
260 (18), the modifications do not influence the colonization of ECM fungi, suggesting that ECM
261 spores are ubiquitously present in the urban environment. However, to our knowledge, studies
262 that explicitly explore the effects of host age on ECM fungal communities in urban soils are
263 non-existent.

264

265 Despite the lack of an overall park age effect on ECM fungal community diversity,
266 community composition responded to park age in the broadleaf tree rhizospheres. Similar
267 responses were absent with the conifer hosts. The lack of this response under conifer trees is
268 surprising, given that soil chemistry changes (lower pH, higher OM, C and N) were
269 particularly pronounced underneath conifers in our parks (18). Previous studies on ECM
270 succession in natural forests suggest some context dependency of community responses to
271 stand age. ECM fungal composition and diversity were insensitive to the age of oak (*Quercus*
272 *ilex*) stands (45), whereas Kvaschenko et al. reported changes in the ECM fungus species
273 composition along an age gradient of managed *Pinus sylvestris* stands (46). Taken together,
274 these suggest that ECM fungal communities are primarily shaped by host-fungus interactions
275 rather than by abiotic habitat conditions such as soil chemistry.

276

277 **Conclusion**

278

279 Our results demonstrate that, in general, ECM fungi respond to land-use type (urban park vs.
280 non-urban forest stands) and to plant functional types within parks and forests. Although
281 ECM fungal richness was marginally greater in control forests than in urban parks, urban
282 parks still hosted rich and diverse ECM fungal communities. ECM fungal community
283 composition differed between the two habitats, but it was the common taxa that varied in
284 abundance without clear taxon replacements, indicating that key ECM fungi remained mainly
285 the same. In parks, ECM fungal community composition differed between conifer and
286 broadleaf trees. Park age also proved to shape ECM fungal community composition, but this
287 was evident under broadleaf trees only. Interestingly, plant functional group effects tended to
288 be amplified in older parks where ECM fungi have had a longer time to interact with tree
289 roots. We conclude that despite the lack of natural pedogenesis and arrested vegetation
290 succession as well as anthropogenic disturbance that includes raking leaves, mowing and
291 trampling, urban parks host a surprisingly diverse set of ECM fungi. Whether these urban
292 ECM fungal communities functionally approximate those in natural forest stands requires
293 further research.

294

295 **Materials and methods**

296

297 **Study area and sampling design**

298

299 The study sites have been described in detail previously (17, 18). Briefly, we selected 41
300 urban parks in the cities of Helsinki and Lahti, southern Finland, and 5 additional control
301 forests in the proximity of Lahti. The urban parks represent different ages: more than 100
302 years old (the oldest parks were established over two centuries ago), 50 ± 10 years old and 10
303 years old, referred to as old, intermediate and young parks, respectively. We considered two

304 plant functional types in these parks: conifer (43.3% of the conifer tree species represents
305 *Picea* spp.; 20% represents *Abies* sp.; 13.3% represents *Pseudotsuga menziesii*; 13.3%
306 represents *Pinus sylvestris* 13.3%; 10% represents *Larix* sp.) and broadleaf (*Tilia x vulgaris*
307 100%) trees. With a few exceptions, conifer and broadleaf trees existed commonly together
308 within a park. Distance between the two tree types was always greater than the height of the
309 tallest tree. The age of plants within each park age class corresponded with park age, except
310 for the young parks where trees are commonly planted as ca. 10 year old saplings at the time
311 of park construction. The ideal experimental design would have included 15 parks per city,
312 represented by five old, five intermediate and five young parks, with both plant functional
313 types present. However, since some parks did not include both plant functional types, we also
314 selected parks with only one plant functional type. This resulted in a total of 41 urban parks
315 and 58 urban sampling locations with 7-11 replicates per park age and plant functional type.
316 Park sizes varied considerably, ranging from ca. 0.1 ha to several hectares, but with no
317 systematic grouping of size with park age and plant functional type.

318

319 Soil sampling and edaphic conditions

320

321 ECM fungi colonize roots, but they grow from roots into the soil to deliver soil nutrients to
322 the roots. Because of this, soil is a good proxy in studying ECM fungal communities and
323 assigning the detected taxa to ecological roles (47-49). We sampled soils in May 2015 at the
324 edge of the canopy projection so that distance to the nearest tree trunk ranged from 1 m
325 (young parks; samples always collected outside the planting pit) to several meters (old parks).
326 At each sampling point, we subsampled 3 soil cores (top 10 cm) using a steel push corer (10
327 cm deep, 2.54 cm diameter), pooled the three subsamples into one composite for a total of 68
328 samples across the experiment (58 urban park samples and 10 control forest samples). The
329 corer was sterilized using 70% ethanol between samples. Samples were stored in Minigrip

330 bags on ice in the field and frozen at -20°C in the laboratory. Before DNA extraction, the
331 samples were thawed at room temperature and sieved through a 2 mm mesh to remove any
332 remaining large particles. The edaphic conditions (0-10 cm deep) of all samples were
333 analyzed in our previous studies (18, 50). Five variables, carbon content (C), nitrogen content
334 (N), organic matter (OM), percentage sand (PS) and pH were used in our statistical analyses.

335

336 DNA extraction, PCR and Illumina MiSeq sequencing

337

338 Total DNA was extracted from ~ 10 g (8.2–10.1 g fresh weight) soil samples using the
339 PowerMax® Soil DNA Isolation Kit (MoBio, Carlsbad, California) following the
340 manufacturer's instructions, and stored at -20°C until PCR amplification. The hypervariable
341 Internal Transcribed Spacer (ITS2) region of the fungal rRNA gene was amplified with
342 primers fITS7 5'-GTGARTCATCGAATCTTTG-3' incorporating 5'-
343 GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3' overhang and ITS4 5'-
344 TCCTCCGCTTATTGATATGC-3' incorporating 5'-
345 ATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-3' overhang. In the secondary
346 PCR, the full-length P5 and indexed P7 Illumina MiSeq adapters were used. The PCR
347 reactions were performed as in (51). The samples were analyzed using the Fragment Analyzer
348 (Advanced Analytical, USA) and amplicons sequenced with Illumina MiSeq (v.3 2x300bp
349 paired-end) at the Institute of Biotechnology, University of Helsinki. The paired fastq files are
350 available in the Sequence Read Archive at NCBI (www.ncbi.nlm.nih.gov) under accession
351 number SRX1584451.

352

353 Bioinformatics

354

355 We extracted the ECM fungus dataset from a broader environmental sequence dataset
356 described in our previous study (17). In the current contribution, we explicitly focused on
357 mycorrhizal communities, because our previous effort on general fungal communities poorly
358 permitted us to address changes in ECM fungal community composition and diversity.
359 Briefly, we processed the paired end sequence data (.fastq) using mothur version 1.36.1(52).
360 The fungal .fastq files were contiged and any sequences with ambiguous bases, with more
361 than one mismatch to the primers, homopolymers longer than 8 bp and any without a
362 minimum overlap of 50 bp were removed. The sequences were screened for chimeras using
363 UCHIME (53) and putative chimeras removed. To permit pairwise alignment of fungal ITS
364 sequences to calculate a pairwise distance matrix, we omitted sequences that were shorter
365 than 300bp, and truncated the remaining sequences to the first 300bp. These fungal sequences
366 were assigned to taxa using the Naïve Bayesian Classifier and the UNITE-curated
367 International Nucleotide Sequence Database reference database (54). Any sequences not
368 assigned to Kingdom Fungi were removed. A pairwise distance matrix was derived from
369 pairwise alignments and sequences clustered to OTUs at a 97% threshold using nearest
370 neighbour joining. All low abundance OTUs were removed (≤ 10 sequences across all
371 experimental units) as they may be PCR or sequencing artifacts (55-57). We assigned OTUs
372 into trophic modes using the FUNGuild database (58) and selected ECM OTUs at the cut
373 value of “highly probability”. This resulted in a total of 216 916 sequences representing 357
374 ECM OTUs. We estimated richness and diversity indices for ECM fungal communities in
375 mothur. Observed OTU richness (S_{obs}), the complement of Simpson’s diversity ($1/D: 1/\sum p_i^2$),
376 and Simpson’s evenness ($E_D: 1/\sum p_i^2/S$), with p_i representing the abundance of each OTU
377 within a sample, were iteratively calculated and subsampled at 517 sequences per sample.

378

379 Statistical analyses

380

381 All statistical analyses were performed in R (version 3.2.1, R Development Core Team, 2015)
382 using various packages.

383

384 ECM fungal community data were analyzed using two different strategies. First, we evaluated
385 differences between urban parks and the control forests (land-use type), using a dataset
386 including the 10 controls (five control forests with conifer and broadleaf species in the
387 vicinity of the city of Lahti) and old parks (five parks with conifer and broadleaf species
388 within the city of Lahti), for a total of 20 experimental units. We compared controls to old
389 parks because they have trees of virtually the same age class, which enabling comparison
390 between habitat types and excluding tree age. In this analysis, we specifically explored
391 differences in ECM fungal communities in control forests and comparable park treatments.
392 Differences in ECM fungal diversity indices (Ln-transformed where necessary) and counts
393 (sequence abundance) of the dominant genera (the 10 most abundant genera) between land-
394 use types were evaluated using generalized linear mixed models (GLMM) with the *lmer* and
395 *glmer* functions in the lme4 package in R. Diversity index data were modeled following a
396 Gaussian distribution, while count data (the dominant genera) were modeled following a
397 Poisson error distribution, with an individual-level random effect included to account for
398 possible overdispersion (59). Predictor variables included plant functional type as a factor,
399 land-use type as a factor and their interaction, as well as C and N content of the soil, OM,
400 percentage sand (PS) and soil pH. Since our samples were from two different vegetation
401 treatments that may locate in the same park, park location was added as a random term. We
402 performed model selection by removing non-significant terms, starting with the term with the
403 highest p-value. C, N, OM, PS and pH were initially subject to model simplification until only
404 terms with p-values < 0.1 were left. If the land-use type x plant functional group interaction
405 remained non-significant (p-values > 0.1) after this procedure, it was also removed. However,
406 to remain true to our experimental design, the main effects (land-use type and plant functional

407 type) were always retained in the model irrespective of their significance. Second, we
408 evaluated the effects of plant functional type and park age on ECM fungi. Here, we analyzed
409 a dataset including all park age categories and plant functional types, but omitted the control
410 stands. Similarly to the above, the response of individual ECM taxa (the 10 most abundant
411 genera only) (count data) and diversity indices (Ln-transformed when necessary) to park age
412 and plant functional type were tested using GLMM. In these analyses, land-use was replaced
413 with park age; otherwise the analyses were identical to those described for the first strategy.

414

415 For each of the two discrete analyses, we also utilized non-metric multidimensional scaling
416 (NMDS, vegan package in R) to visualize community wide responses to the factors included,
417 based on Bray-Curtis dissimilarity. Soil carbon and nitrogen content, OM, PS and soil pH
418 were correlated with the community structure using permutation tests as the vector fitting
419 procedure (the *envfit* function in vegan). We did the same ordination analyses on the park
420 ECM fungal communities under conifer and broadleaf trees separately. These analyses were
421 motivated by the distinctions between the two plant functional types, thus permitting a more
422 detailed focus on the effects of park age and multi tree species within the conifer group. In
423 addition, because the same conifer tree species were not consistently present in our parks, we
424 included 5 tree species in this plant functional group and tested the tree species effect on the
425 ECM fungal communities. Although the five conifer tree species differed in their ECM fungal
426 communities ($r^2 = 0.110$, $p = 0.029$, Fig. 5) when comparing “inter-tree type variation” with
427 variation between the two plant functional types, the tree species effect within the conifer
428 group was minor.

429

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431

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436

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611

612 **Figure legends**

613

614 **Fig. 1** Predicted (mean \pm SE) ECM fungal OTU richness (a), diversity (b) and evenness (c)
615 (GLMM results) in parks (both Lahti and Helsinki parks, left side panels) across plant
616 functional types (symbols) and park ages (x-axis), and between old Lahti parks and control
617 forests (right side panels) across plant functional types.

618

619 **Fig. 2** NMDS plots for ECM fungal communities. ECM fungal communities (a) of soils
620 below broadleaf and conifer trees in old Lahti parks and control forests; ECM fungal
621 communities (b) of soils below broadleaf trees in parks. Statistically significant ($p < 0.05$)
622 vectors (soil pH, %N, %C, organic matter and percentage sand) are shown. All NMDS plots
623 showed significant differences ($p < 0.05$) either across land-use type (control forest vs. urban
624 park), plant functional type or park age by *envfit* analyses.

625

626 **Fig. 3** Predicted count (mean \pm SE) of the ten most abundant ECM fungal genera across land-
627 use type (control forest vs. old urban park) and plant functional type (GLMM results).

628

629 **Fig. 4** Predicted count (mean \pm SE) of the ten most abundant ECM fungal genera across plant
630 functional type and park age (GLMM results).

631

632 **Fig. 5** NMDS plot for ECM fungal communities under conifer trees in parks. The NMDS plot
633 shows significant differences ($p < 0.05$) across conifer tree species by *envfit* analyses.

634

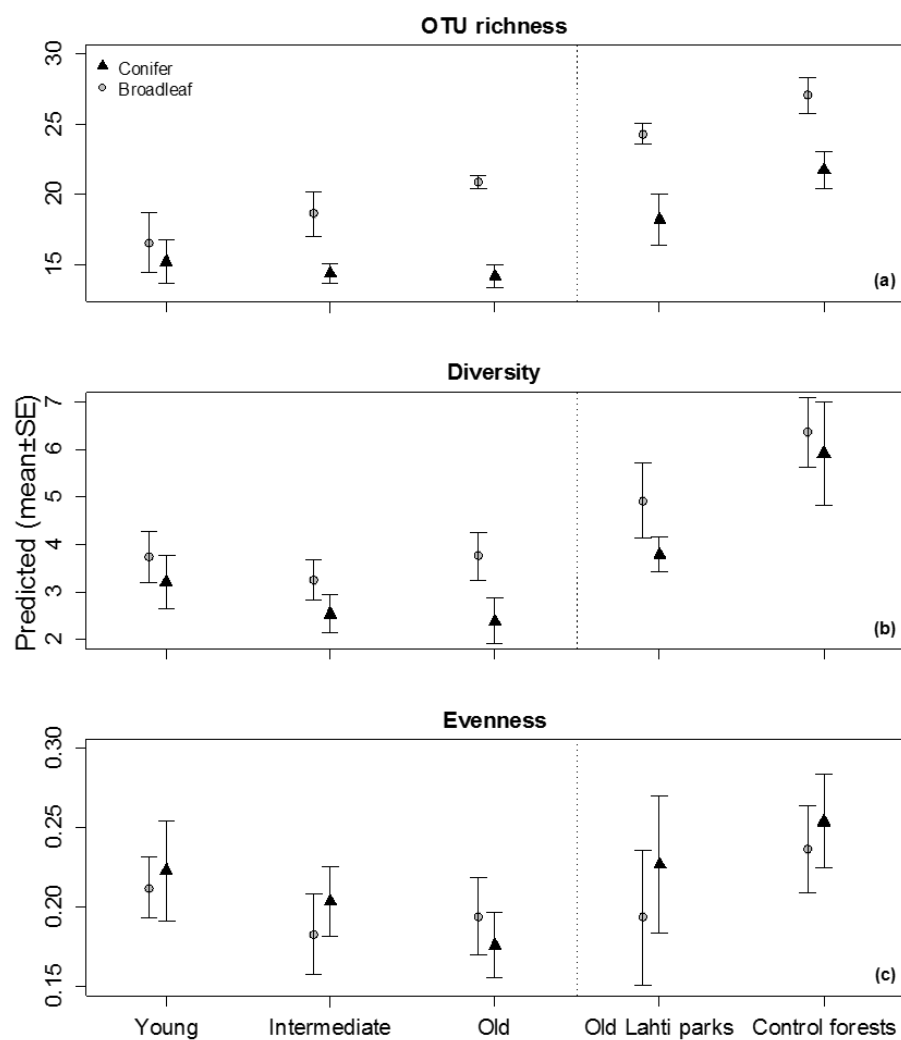


Fig. 1 Predicted (mean \pm SE) ECM fungal OTU richness (a), diversity (b) and evenness (c) (GLMM results) in parks (both Lahti and Helsinki parks, left side panels) across plant functional types (symbols) and park ages (x-axis), and between old Lahti parks and control forests (right side panels) across plant functional types.

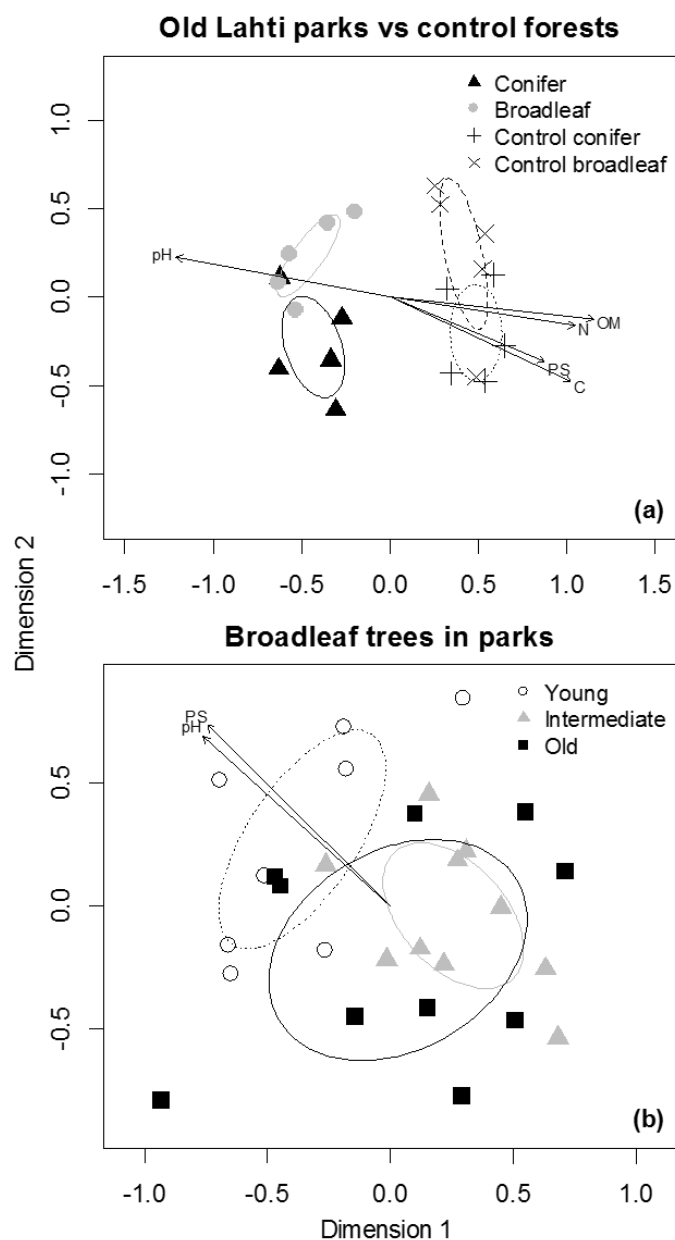


Fig. 2 NMDS plots for ECM fungal communities. ECM fungal communities (a) of soils below broadleaf and conifer trees in old Lahti parks and control forests; ECM fungal communities (b) of soils below broadleaf trees in parks. Statistically significant ($p < 0.05$) vectors (soil pH, %N, %C, organic matter and percentage sand) are shown. All NMDS plots showed significant differences ($p < 0.05$) either across land-use type (control forest vs. urban park), plant functional type or park age by *envfit* analyses.

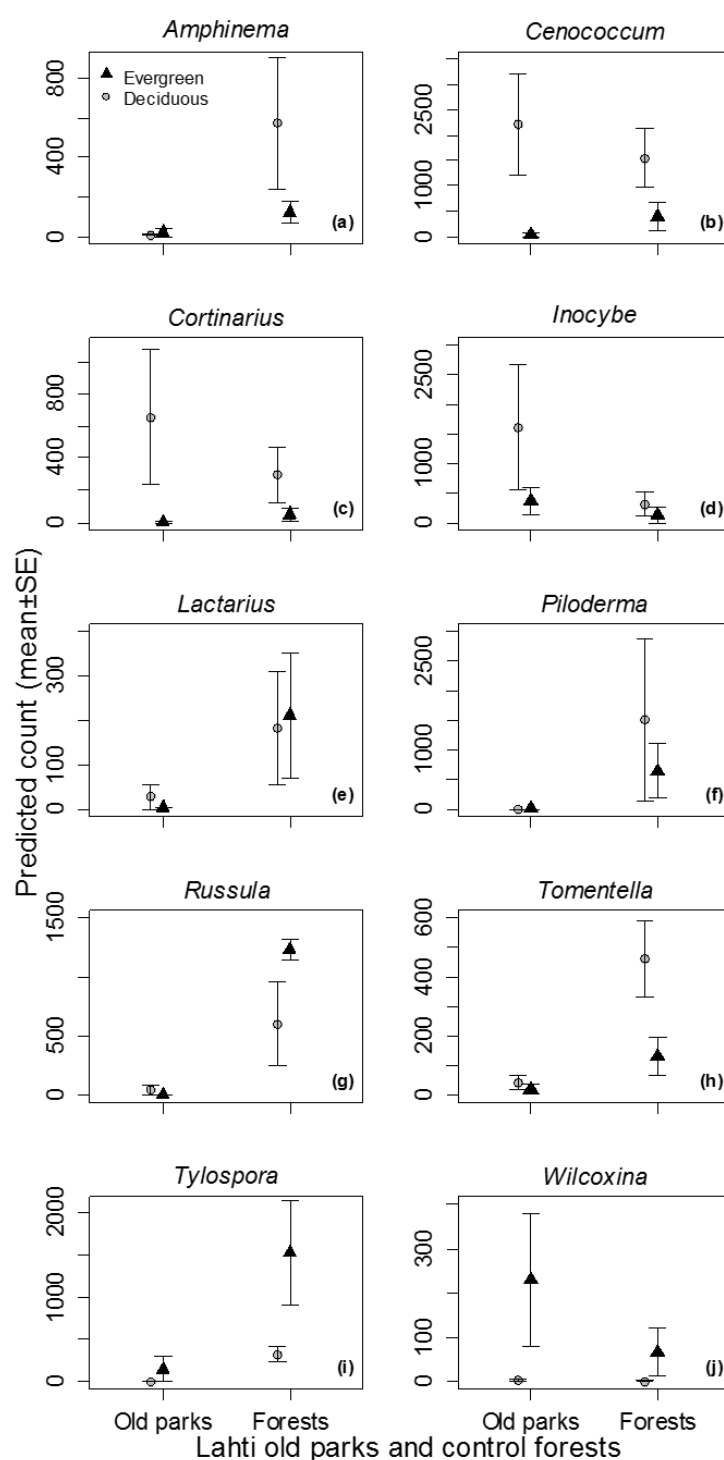


Fig. 3 Predicted count (mean \pm SE) of the ten most abundant ECM fungal genera across land-use type (control forest vs. old urban park) and plant functional type (GLMM results).

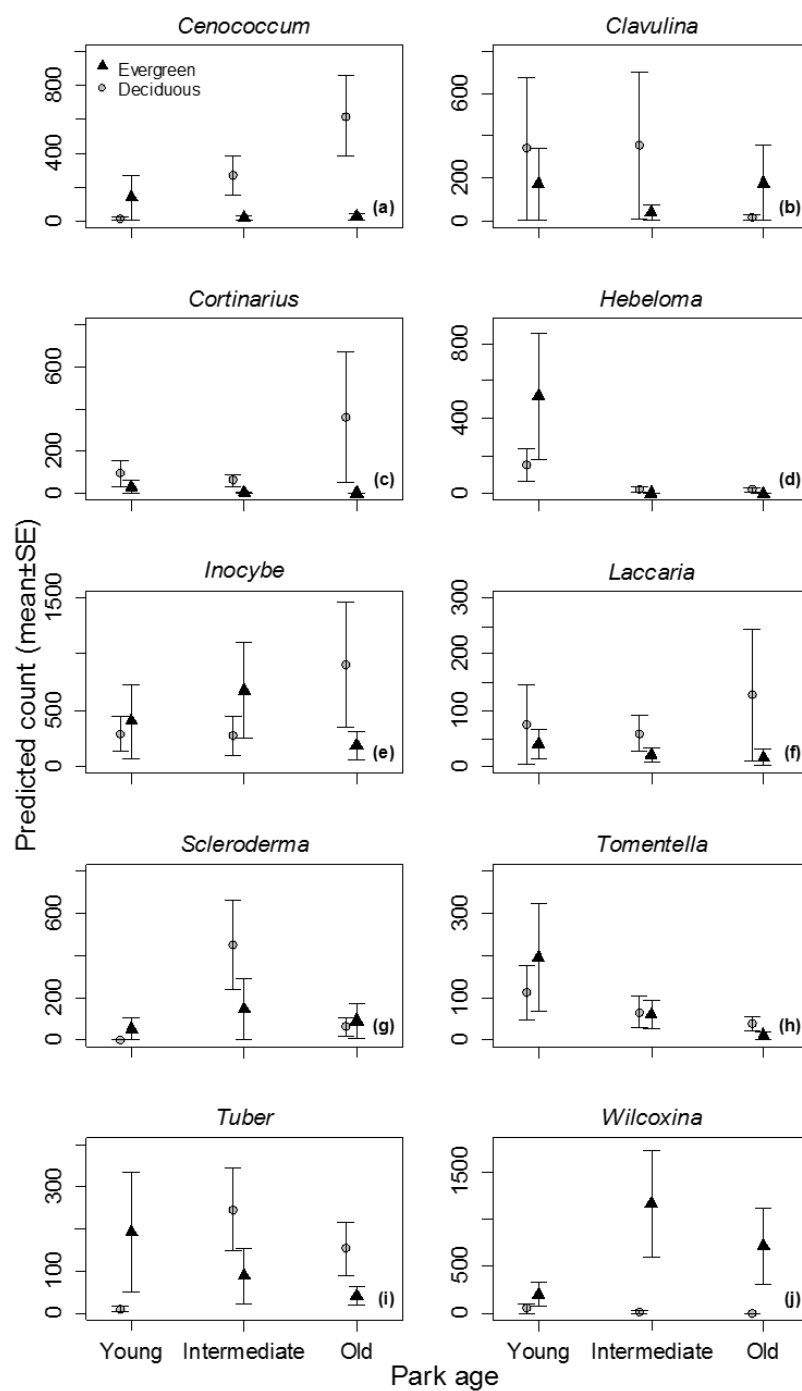


Fig. 4 Predicted count (mean \pm SE) of the ten most abundant ECM fungal genera across plant functional type and park age (GLMM results).

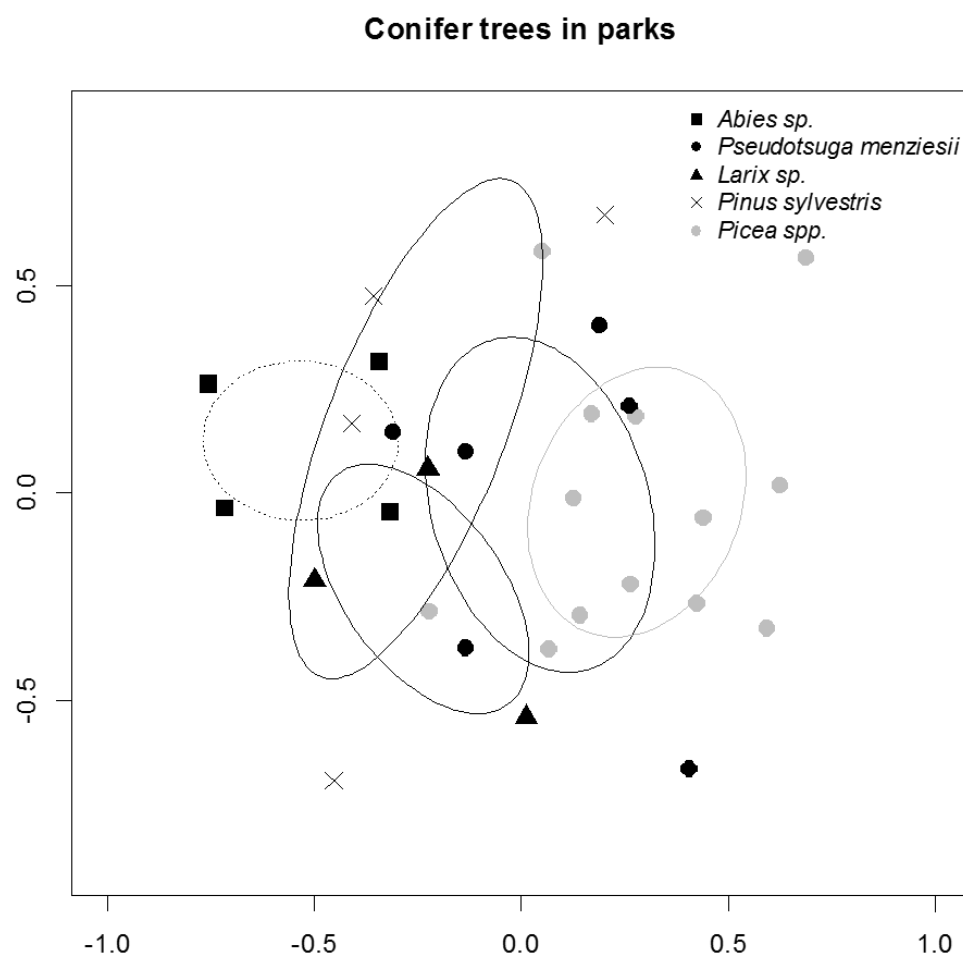


Fig. 5 NMDS plot for ECM fungal communities under conifer trees in parks. The NMDS plot shows significant differences ($p < 0.05$) across conifer tree species by envfit analyses.